

Expression of Fas Antigen (CD95) on Human Leukemic Cells and Assessment of Apoptosis on Fas+ and Fas- Samples by Flowcytometry Method

Tahereh Zandieh, Asghar Safari Fard, and Mahnaz Aghaipur

Department of Research Center of Iranian Blood Transfusion
Organisation Tehran, Iran

ABSTRACT

Regulation of normal cell growth and turnover is balanced between cell proliferation, cell differentiation and apoptosis.

A disruption of this balance is thought to be an important event leading to carcinogenesis. One of the effector molecules in apoptosis is Fas antigen. Cross-linking of Fas by its ligand (Fas L) or agonistic anti Fas antibodies induces apoptosis of cells expressing Fas on the membrane by triggering cascade of caspases.

The aim of this research was to study the percent of expression of Fas antigen on bone marrow and peripheral blood cells in 100 patients suffering from acute lymphoid and myeloid leukemia by flow cytometry method. Samples were obtained at the time of diagnosis before antileukemic therapy. Expression of Fas antigen on normal control peripheral leukocytes was also analysed.

From these data, it was found that Fas antigen is expressed in all cases, but the expression level varied widely.

The percentage of Fas antigen expression in all of acute lymphoid leukemia samples was below 20%, but in acute myeloid leukemia samples, 8 out of 50 cases was above 20%. In normal control samples, the mean value for monocytes was higher than granulocytes and in granulocytes higher than lymphocytes.

Expression of Fas antigen in most of the leukemic cells was low and the preliminary results showed that increase in Fas antigen expression above 20% after treatment, is a favorable prognostic sign associated with increase relapse free and total survival. Thus evaluation of this antigen before, during and after treatment is recommended.

Key words: Apoptosis, Fas Antigen, Flowcytometry, Leukemia.

INTRODUCTION

Leukemia is a generalized neoplastic proliferation of leukocytes with or without involvement of the pe-

ripheral blood. Peripheral blood abnormalities and infiltration of non hematopoietic tissues are frequently but not invariably present.¹

Congenital or acquired defects in the apoptotic process may contribute to the pathogenesis of malignant disease.²

Apoptosis occurs in all multi cellular organisms as a means to balance cell proliferation in continu-

*Corresponding Author: Dr. Tahereh Zandieh, Iranian Blood Transfusion Organisation Tehran-Iran. P.O:4686, Tel: (+98 21) 828 8581-3 Fax: (+98 21) 8270001 Email: tz7892000@yahoo.com.

ously renewing tissues in order to maintain a constant organ size. In the hematopoietic system, cell production is delicately balanced against cell death and removal through the monocyte-macrophage system.³ Cell death can also be regulated by proteins that may stimulate apoptosis.

Physiological apoptosis can be induced by a multitude of stimulants, including withdrawal of growth factors, hormones, and activation of death receptors such as CD95 (APO-1/Fas) and tumor necrosis factor-receptor type 1 (TNF-R1).^{4,5}

Fas antigen (also known as Apo-1 or CD95) is a cell surface molecule that triggers apoptosis in susceptible cells upon binding by its ligand (Fas L), or anti-Fas antibody.

The CD95 receptor/CD95 ligand (CD95/CD95L) system has been described as a key signal pathway involved in regulation of apoptosis in several cell types.⁶ The CD95, a 48 KD type I-transmembrane receptor is a membrane of the tumor necrosis factor/nerve surface molecules, which includes various molecules involved in immune regulation, such as TNF receptor I and II, CD27, CD30 and CD40.⁷

Crosslinking by agonistic antibodies or the natural ligand activates a signal cascade via FADD/MORT-1 and FLICE/MACH (caspase-8) that directly leads to activation of ICE/ced-3 protease (caspases) which function as downstream effectors of cell death. The Fas-based death pathway plays an important regulatory role for the elimination of cells in vivo, and is believed to play a key role in the elimination of malignant cells.

Some researchers demonstrated that Fas was expressed on majority of human leukemia cells, although the intensity of expression was variable.⁸ The crosslinking of Fas with anti-Fas monoclonal antibody could induce apoptotic cell death in certain cases of leukemia. However, in half of Fas-expression leukemia cases, cell growth was not inhibited with the treatment of anti-Fas.⁹ Moreover, normal mature B-cells from peripheral blood are negative for Fas.¹⁰ As Fas expression status on leukemia and lymphoma B-cells from different disease entities is controversial, this study was conducted to examine quantitative Fas expression levels on blastic cells (BM or PB) in 100 patients suffering from acute lymphoid as well as myeloid leukemia in various stages of maturation and differentiation of B-cell ontogeny, and to find out whether, evaluation of Fas antigen before, during and after treatment is a favorable prognostic sign associated with increase relapse free and total survival.

MATERIALS AND METHODS

Peripheral blood (PB) or bone marrow (BM) at

onset of these malignancies was taken from 50 cases (20 women and 30 men, mean age 54 ± 4) of acute lymphoblastic leukemia (ALL) and 50 cases (18 women and 32 men, mean age 53 ± 6) of acute myeloblastic (AML) and 50 healthy controls (26 women and 24 men, mean age 38 ± 6) for the present study. None of the patients had received any treatment prior to investigation. The diagnosis of the patients studied was assessed hematocytologically, morphologically and immunologically, according to the FAB classification of acute leukemia. Fas was detected by flow cytometry in fresh peripheral blood mononuclear cell from EDTA treated blood and in fresh bone marrow. We examined leukemic samples from subgroup of M0 to M5, but we had not any samples from M6 and M7.

Mononucleated cells were isolated by Ficoll-Hypaque density centrifugation. The cell suspension contained $>50\%$ blast cells as assessed by may-gimsa morphology, in-patients.

Flow cytometry analysis and quantification of Fas antigen:

Flow cytometric analysis of Fas expression was performed using Coulter Epics XL-USA flow cytometer. In brief, 1000,000 cells (10mL) were incubated with 10 mL fluorescein-isothiocyanate conjugated anti Fas (CD95) monoclonal antibody (MoAb) for 30 min at $2-8^{\circ}\text{C}$. After washing with phosphate buffered saline (PBS), the intensity of fluorescence was analyzed with flow cytometry.^{11,12} Isotype matched control MoAb was used to determine nonspecific binding. The percentage of Fas antigen expression, which was higher than 20% was considered as positive.

Statistical analysis

Comparison between groups were made by the LSD test (post hoc), using the SPSS version II software package. A p value lower than 0.05 was considered significant.

RESULTS

The membrane molecules were expressed at various levels in cell subtypes of acute leukemia tested.

Evaluation of CD95 in healthy control

In this evaluation percentage of CD95 on membrane of granulocytes, lymphocytes and monocytes in healthy control were determined as shown in Fig. 1. There was significant difference between 3 sub groups of cells in healthy control ($p < 0.05$). The percentage of CD95+ in monocytes was higher than granulocytes and in granulocytes higher than lymphocytes (monocytes 26.6 ± 0.7 , granulocytes 19.1 ± 3.6 , and lymphocytes 16 ± 8.046).

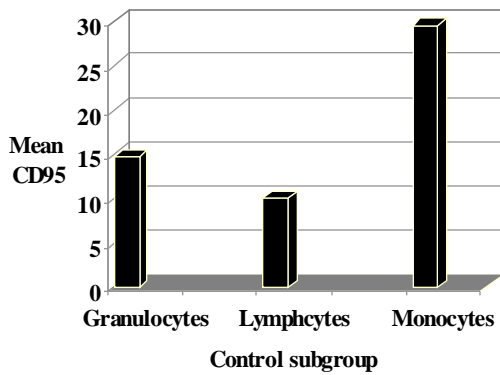
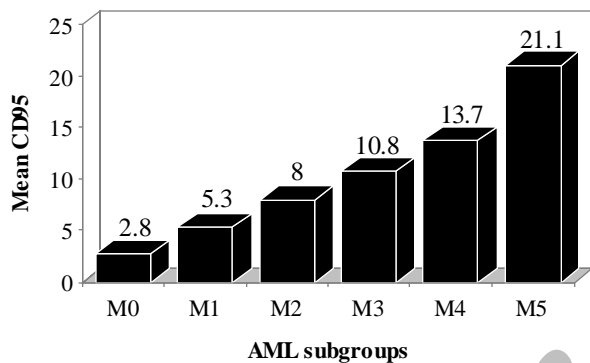


Figure 1. Percentage of CD95 in granulocytes, lymphocytes and monocytes in healthy control.



$P < 0.05$

Figure 2. Percentage of CD95 in different subtype of AML.

Evaluation of CD95 in AML

50 patients were selected before treatment according to the FAB subtype. Percentage of CD95 membrane was determined in these patients. 42 out of 50 AML cases showed less than 20% CD95 expression (Fas-) in comparison to 8 cases (8 out of 50) of all subtypes, which expressed more than 20% CD95 (Fas+). As shown in Fig. 2, there was significant meaningful difference between all subtypes of AML group (M1-M5) ($p < 0.05$).

Among AML class M5 had the highest expression level (46.2%), thus more mature M5 subtypes revealed a significantly higher CD95 expression than immature subtypes. There was no significant association between CD95 and other parameters such as age, sex, and WBC count. (Figure 3 and 4)

Evaluation of CD95 in ALL

50 patients were selected before treatment accord-

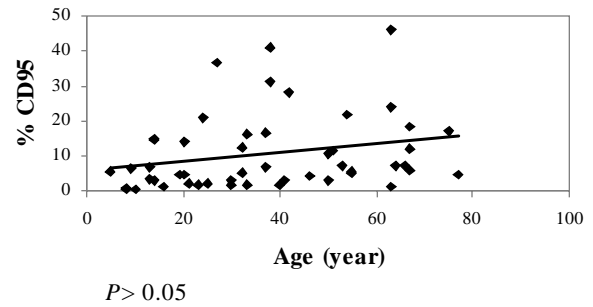


Figure 3. Association between percentage of CD95 and age in AML

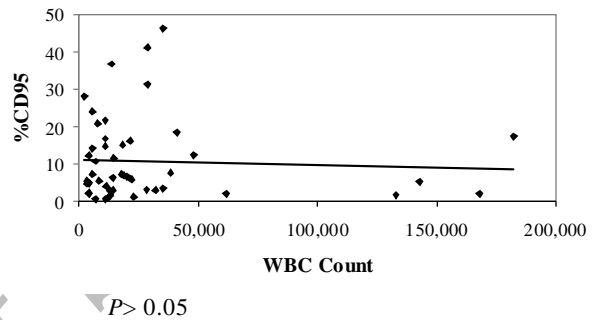
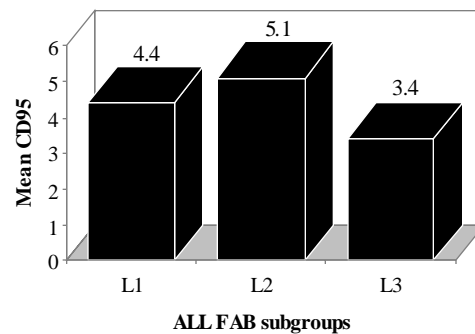


Figure 4. Association between percentage of CD95 and WBC in AML.



$P < 0.05$

Figure 5. Percentage of CD95 in subtype of ALL.

ing to the FAB subtype. Percentage of membrane CD95 was determined in these patients. All cases of ALL showed less than 20% CD95 expression (Fas-). There was no significant meaningful difference between all subtypes (Fig. 5).

As shown in Figures 6 and 7 there was no meaningful association between CD95 and other parameters such as age, sex, and WBC count ($p > 0.05$).

Expression of Fas antigen on leukemia cells

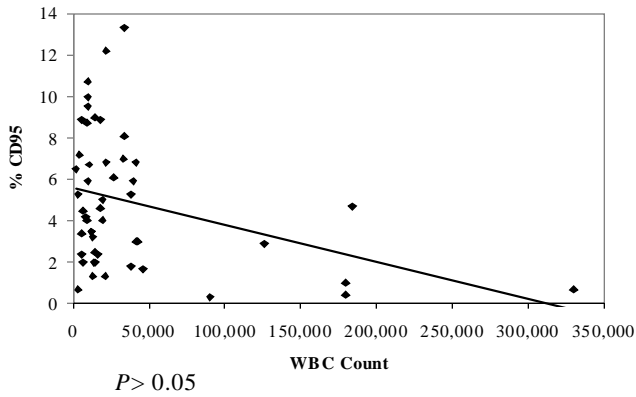


Figure 6. Association between percentage of CD95 and WBC in ALL.

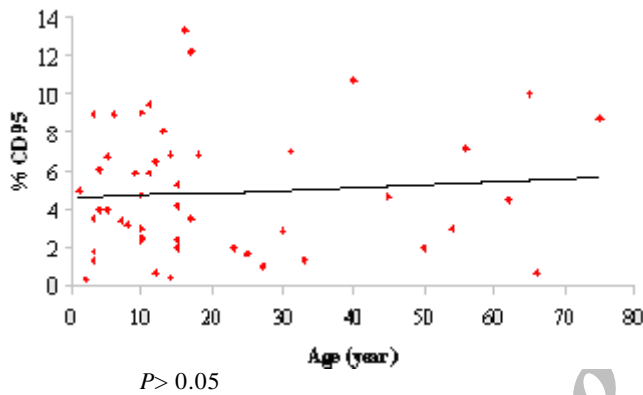


Figure 7. Association between percentage of CD95 and age in ALL.

DISCUSSION

The growth and death of leukemia cells are modulated by the external signals that induce apoptosis via the cell surface molecules. The Fas receptor has emerged as an important cellular component that mediates apoptotic cell death in leukemic cells.¹² Apoptosis by the Fas-triggering system is now accepted as a critical element in the repertoire of potential cellular responses.¹³

Vast investigations have been conducted on expression of Fas antigen on cancer patients, especially on leukemia. But there is different opinions about CD95 and malignancy. This research is the first study in Iran, in which we examined the expression of CD95 on AML and ALL patients, and compared them with healthy control. CD95 antigen over 20% of cell was expressed on the cell surface in 8 out of 50 cases and below 20% of cells in 42 out of 50 cases of AML patients.

Our data showed that the expression of Fas was variable in patients with AML. Similar with the finding of others¹⁴ we observed more mature M5 subtypes revealing a significantly higher CD95 expression than immature FAB subtype.

Some authors, concluded that the different CD95 expression and CD95 function of AML cells resembled the maturation-dependent increase in CD95 expression and CD95 function of normal myeloid progenitor cells¹⁵ CD95 is expressed in many normal as well as malignant human cells, including T-cell leukemia, B-cell leukemia¹⁶ CD95 expression has been associated with a better prognosis in B-cell lymphoma and with responsiveness to chemotherapy in AML,¹⁷ whereas most primary T-cell leukemias are constitutively resistant against CD95 induced apoptosis.¹⁸ In the present study we investigated CD95 in ALL patients, and we observed CD95 antigen below 20% in all ALL cases, before treatment. There was no difference between subtype cases, which is in accordance with the finding of others¹⁹ and the early reports showed that increase in Fas antigen expression above 20% after treatment was a favorable prognostic sign associated with increased relapse free and total survival.

Thus evaluation of this antigen before, during and after treatment is recommended, because the determination of Fas antigen may help to predict the susceptibility to trigger apoptosis by cytotoxic chemotherapy. Fas antigen positively was not associated with other parameters such as age, sex and WBC count.

CD95 expression in monocytes was higher than granulocytes and in granulocytes was higher than lymphocytes, which is in accordance with others.¹⁴

In summary, Fas is heterogeneously expressed on leukemia and lymphoma cells, and may be a biological marker for characterizing B-cell neoplasm by quantitative analysis, since proliferation and apoptosis of blast cell in acute leukemia play a major role in determining the response to therapy. However evaluation of CD95 expression, and induction of apoptosis in CD95 system could be a novel and effective approach not only for treatment but also for prevention of relapse in ALL and AML. Further studies will be required to evaluate the biological role of Fas, for example;

- 1-Evaluation of high number of CD95+ cells in leukemic cases with different monoclonal antibodies.
- 2- Evaluation of CD95 in different stages of treatment.
- 3-Evaluation of different parameters in apoptosis.
- 4-Evaluation of apoptosis inhibition and comparing it with Fas antigen.

ACKNOWLEDGEMENT

We would like to acknowledge the help of Miss. A.

Tababaiian, M. Nikogoftar, and BabaHosseini.

REFERENCES

1. Henry JB. Clinical diagnosis and management by laboratory methods. New York: Sunders company, 2001: 586-662.
2. Thompson CB. Apoptosis in the pathogenesis and treatment of disease. *J Sci* 1995; 267:1456-1462.
3. William WJ, Beutler AJ. Hematology. Baltimore: William and Wilkins, 1993: 377-400.
4. Friesen C, Fulda S, Debatin KM. Cytotoxic drugs and the CD95 pathway. *Leukemia* 1999; 13(11):1854-8.
5. Belaud-Rotureau MA, Durrieu F, Labroille G, Lacombe F, Fitoussi O, Agape P, et al. Study of apoptosis-related responses of leukemic blast cell to in-vitro anthracyclin treatment. *Leukemia* 2000; 14(7):1266-75.
6. Krammer PH, Dhein J, Walczak H, Behrmann I, Mariani S, Matiba B, et al. The role of APO-1 mediated apoptosis in the immune system. *Leukemia* 1994; 142:175-91.
7. Klas C, Debatin KM, Jonker RR, Krammer PH. Activation interferes with the APO-1 pathway in mature human T cells. *Int Immunol* 1993; 5(6):625-30.
8. Takahashi T, Tanaka M, Inazawa J, Abe T, Suda T, Nagata S. Human Fas ligand: gene structure, chromosomal location and species specificity. *Int Immunol* 1994; 6:1567-74.
9. Inaba H, Komada Y, Li QS, Zhang XL, Tanaka S, Azuma E, et al. mRNA expression of variant Fas molecules in acute leukemia cell. *Am J Hematol* 1999; 62(3):150-8.
10. Daniel PT, Krammer PH. Activation induces sensitivity toward APO-1 (CD95)-mediated apoptosis in human B cells. *J Immunol* 1994; 152(12):5624-32.
11. Poncelet P, Carayon P. Cytofluorometric quantification of cell-surface antigens by indirect immunofluorescence using monoclonal antibodies. *J Immunol Methods* 1985; 85(1):65-74.
12. Adrienne MG, Afshin S, Arian JM. Use of flowcytometry techniques in studying mechanisms of apoptosis in leukemic cells. *Cytometry* 1997; 29: 97-105.
13. Yoshihiro K, Yan WZ, Xiao LZ. Fas/APO1 (CD95)-mediated cytotoxicity is responsible for the apoptotic cell death of leukemic cells induced by interleukin-2-activated T cells. *Br J Hematol* 1997; 96:147-57.
14. Wuchter C, Karawajew L, Ruppert V, Buchner T, Schoch C, Haferlach T, et al. Clinical significance of CD95, Bcl-2 and Bax expression and CD95 function in adult de novo acute myeloid leukemia in context of P-glycoprotein function, maturation stage, and cytogenetics. *Leukemia* 1999; 13(12):1943-53.
15. Takenaka K, Nagafuji K, Harada M, Mizuno S, Miyamoto T, Makino S, et al. In vitro expansion of hematopoietic progenitor cells induces functional expression of Fas antigen (CD95). *Blood* 1996; 88(8):2871-7.
16. Karawajew L, Wuchter C, Ruppert V, Drexler H, Gruss HJ, Dorken B, et al. Differential CD95 expression and function in T and B lineage acute lymphoblastic leukemia cells. *Leukemia* 1997; 11(8):1245-52.
17. Min YH, Lee S, Lee JW, Chong SY, Hahn JS, Ko YW. Expression of Fas antigen in acute myeloid leukaemia is associated with therapeutic response to chemotherapy. *Br J Haematol* 1996; 93(4):928-30.
18. Lucking-Famira KM, Daniel PT, Moller P, Krammer PH, Debatin KM. APO-1 (CD95) mediated apoptosis in human T-ALL engrafted in SCID mice. *Leukemia* 1994; 8(11):1825-33.
19. Beltinger C, Kurz E, Bohler T, Schrappe M, Ludwig WD, Debatin KM. CD95 (APO-1/Fas) mutations in childhood T-lineage acute lymphoblastic leukemia. *Blood* 1998; 91(10):3943-51.